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Measurement of airborne fungal spore dispersal from three types of flooring materials

Mark P. Buttner[1], Patricia Cruz-Perez[1], Linda D. Stetzenbach[1*], Paula J. Garrett[1] & Alan E. Luedtke[2]

[1]*Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas, 4505 S. Maryland Parkway, Las Vegas, Nevada 89154-4009, USA;* [2]*E.I. DuPont de Nemours and Co., 125 Town Park Drive, Suite 400, Kennesaw, Georgia 30144, USA (***author for correspondence, E-mail: STETZENL@UNLV.EDU)*

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Abstract

Research was conducted in an experimental room to measure the effect of human activity on airborne dispersal of settled fungal spores from carpet and vinyl tile flooring. A series of experiments were conducted in which commercial loop pile carpet, residential cut pile carpet, or vinyl tile installed in the experimental room were contaminated with *Penicillium chrysogenum* spores. The flooring materials were contaminated to two different levels (10^6 and 10^7 colony forming units per square meter [c.f.u./m^2] of flooring surface). Airborne culturable and total *P. chrysogenum* concentrations were measured using Andersen single-stage impactor samplers and Burkard personal slide impactor samplers, respectively. Bioaerosol concentrations were measured at floor level, 1 meter, and the adult breathing zone (1.5 meter) heights before and after human activity consisting of walking in a prescribed pattern for 1 minute in the room. Airborne *P. chrysogenum* concentrations were greater with the higher surface loading for all three flooring materials. For all flooring materials there was no significant difference between sampler locations, although the data from the 1-meter location were the highest, followed by the floor level and the breathing zone locations, respectively. The data from these experiments indicate that while a very small fraction of culturable *P. chrysogenum* spores present on flooring materials were aerosolized by walking, relatively high airborne concentrations of spores maybe re-entrained from contaminated materials. The airborne *P. chrysogenum* concentrations were significantly higher after walking on cut pile carpet than with the other two flooring materials at both contamination levels, with the differences in concentration often ≥ 2 orders of magnitude. No differences were measured in airborne culturable *P. chrysogenum* between vinyl flooring and loop pile carpet at both contamination levels. Total spore data from the experiments with the 10^7 c.f.u./m^2 contamination level indicated that walking on loop pile carpet produced higher airborne spore concentrations than similarly contaminated vinyl tile although no significant difference was observed at the 10^6 c.f.u./m^2 level.

1. Introduction

Biological contamination of indoor environments can result in a variety of adverse health effects including allergic reactions and infectious disease (Gravesen, 1978; Burrell, 1991; Gold, 1992). Fungal and bacterial amplification on building surfaces as a result of water damage or improper humidity control have been often implicated as a cause of illness in homes, schools, and offices (Lacey and Crook, 1988; Dales et al., 1991; Wickman et al., 1992). Dust has been associated with a variety of non-specific health effects in buildings (Skov et al., 1990; Nordback et al., 1990; Harrison et al., 1992; Hodgson et al., 1992) and dust may contain fungal spores, pollens, dust mite allergen, animal danders, bacteria, cellulosic fibers, and inorganic particles (Dybendal et al., 1989; Seltzer, 1995). Flooring materials and other interior surfaces

serving as reservoirs for dust have been associated with adverse health effects (Anderson, 1969; Kozak et al., 1980; Nordback and Torgen 1989; Norbeck et al., 1990; Wickman, et al., 1992). The three-dimensional structure of carpet provides the capacity to collect contaminants at higher levels per unit area than hard surfaces (Gravesen et al., 1986; Dybendal et al., 1989; Skov et al., 1990; Dybendal and Elsayed, 1992; Thatcher and Layton, 1995; Zock and Brunekreef, 1995). Monitoring studies have been conducted in hospitals comparing bacterial contamination levels in rooms with carpeting versus tile flooring (Shaffer and Key, 1966; Litsky, 1973; Rylander et al., 1974; Bakker and Faoagali, 1977; Anderson et al., 1982). However, the data attempting to relate surface contaminant levels with airborne contaminant levels in these and other studies were variable (Gravesen, et al. 1986; Price et al., 1990; Zock and Brunekreef, 1995; Kildeso et al., 1999). The comparison of bioaerosol levels in indoor environments with various surfaces has been confounded by a variety of uncontrolled factors (e.g., external conditions, internal conditions of ventilation rate and humidity, building occupant activities, building maintenance practices, and sampling techniques). While the dispersal of fungal spores from contaminated carpet by human activity in a controlled laboratory environment has been demonstrated, surface contamination measurements were only semi-quantitative (Buttner and Stetzenbach, 1993). Therefore, research was conducted to evaluate the re-suspension of settled fungal spores from flooring materials with known surface contamination levels. This study measured airborne fungal spores released from purposefully contaminated sheet vinyl, loop pile carpet, and cut pile carpet as a result of disturbance by walking. Additional information was obtained under more aggressive disturbance conditions, which involved wet mopping the vinyl and vacuuming the carpets. The research was performed under the controlled conditions of an experimental room with a selected fungal spore in the respiratory-relevant size range of ca. 2–3 μm. Fungal contamination levels of flooring materials were selected based on prior experience in this experimental setting (Stetzenbach and Buttner, 1993) and biocontamination levels measured in field studies (Anderson, 1969; Anderson et al., 1982; Stetzenbach, unpublished data). Airborne culturable measurements were compared with measurements of surface loading concentrations. In addition, total airborne spore concentrations were measured.

2. Materials and methods

2.1 *Test organism and culture medium*

Spores of the fungus *Penicillium chrysogenum* (obtained from Harriet Burge, School of Public Health, Harvard University, USA) were used as the test organism. This fungus, which is a common isolate in indoor air samples, produces spores that are spheroidal to ellipsoidal in shape with a diameter of 1.8 to 3.5 μm (Buttner and Stetzenbach, 1993). To obtain spores for the experiments, *P. chrysogenum* was cultured on malt extract agar (MEA, Difco Laboratories, Detroit, Michigan, USA) and incubated at 23 °C for 30 days. Spores were harvested from the agar plates and stored dry at 4 °C until needed. During the experiments, all samples collected on MEA plates were incubated at 23 °C for a minimum of 3 days.

2.2 *Experimental room*

An experimental room designed to resemble a residential indoor environment was used in this study (Fig. 1; Buttner and Stetzenbach, 1993). The room measures 4.0 m by 4.0 m by 2.2 m high. A sheet vinyl floor was installed prior to the start of these experiments and the gypsum board ceiling and walls were coated with interior latex paint. The room was equipped with a heating, ventilation, and air conditioning (HVAC) system sized to simulate a residential system, with 13 $\times$ 20 cm rectangular bare metal ductwork. The room was operated as a closed system with two 10 $\times$ 20 cm registers located at a height of 1.8 m off the floor and 1.8 m apart, supplying HEPA-filtered (99.97% efficiency) air, and one 25 $\times$ 30 cm return vent located 30 cm off the floor on the opposite wall. Dry spores were conveyed into the room through the HVAC system operated with an airflow rate of 4.2 m^3/minute, providing 8.6 air exchanges per hour and a duct velocity of approximately 2.8 m/second. The room was maintained at a positive static pressure (0.02 inches of water) during operation of the HVAC system to minimize contamination from the surrounding area into the room. An anteroom equipped with a HEPA-filtered air shower attached to the room entrance reduced mixing of air on entry and exit of the room during experiments. Temperature was monitored by 20 Type T thermocouples (Thermo Electric Co., Saddle Brook, New Jersey, USA), and relative humidity (RH) was monitored by five RH probes (Hy-Cal Engineering,

Table 1. Experimental design matrix for trials comparing airborne fungal spore concentrations generated by walking on 3 flooring materials at 2 contamination levels

Flooring material	Surface contamination level (c.f.u./m^2)	Number of trials	Number of air samples per trial (pre/post-walking)		Number of surface samples
			Culturable spores	Total spores	
Vinyl tile	10^6	3	3/3	3/3	6
	10^7	3	3/3	3/3	6
Commercial carpet	10^6	3	3/3	3/3	6
	10^7	3	3/3	3/3	6
Residential carpet	10^6	3	3/3	3/3	6
	10^7	3	3/3	3/3	6

El Monte, California, USA) located within the room. Technicians wore a full-face respirator and nonwoven protective clothing for all activities conducted in the contaminated experimental room. Upon completion of each series of experiments, the interior surfaces of the room were disinfected.

2.3 *Experimental design*

A total of 18 experimental trials were performed to evaluate the levels of airborne fungal spores generated from flooring materials associated with walking (Table 1). Six trials were conducted for each of the 3 flooring materials: sheet vinyl, loop pile carpet and cut pile carpet. Two surface contamination levels were tested with three walking trials conducted per flooring material per contamination level. At the end of the walking trials with the highest surface loading, the flooring materials were subjected to a more aggressive disturbance of wet mopping (sheet vinyl) or vacuuming (loop and cut pile carpet). Bulk samples were collected before each trial to quantify the level of surface contamination measured as the number of colony forming units per square meter (c.f.u./m^2) of flooring material. Air samples were collected before and after each activity. These values were recorded as the number of culturable (c.f.u./m^3) and total (spores/m^3) *P. chrysogenum* spores per cubic meter of air sampled.

2.4 *Flooring materials*

Flooring materials were obtained from DuPont Nylon Flooring (Kennesaw, Georgia, USA) and represented readily available retail products. The sheet vinyl design was appropriate for either residential or light commercial use, the loop pile carpet represented a construction generally used in office or school environments, and the cut pile carpet was typical of residential use. All flooring materials were installed over the entire flooring surface of the experimental room by a professional installer. After experiments with the sheet vinyl, the room was disinfected and a loop pile carpet was installed in the room. After completion of the series of experiments with the loop pile carpet, the contaminated carpet was removed and the interior surfaces of the room were disinfected. A cut pile carpet was then installed over a 7/16, 6 lb. bonded urethane pad. Small pre-cut squares of identical flooring material were placed on top of the respective flooring to serve as patches for quantifying surface contamination levels during the experiments (see Assessment of Flooring Contamination).

2.5 *Contamination of flooring materials*

For each series of experiments, *P. chrysogenum* spores were introduced into the room via the air supply duct using a Pitt 3 dry aerosol generator (Weyel et al., 1984; Buttner and Stetzenbach, 1993) and allowed to settle onto the floor. The Pitt 3 produced a dry aerosol of spores by acoustic vibration, which was transported by filtered, dry CO_2 gas from the sealed plexiglass column to the supply duct at a rate of approximately 10 liters per minute. Airborne spore concentrations in the room were measured by an aerodynamic particle sizer (APS, TSI, Inc., St. Paul, Minnesota, USA) positioned inside the room. The APS is capable of real-time measurements of particles in the 0.5 to 30 μm size range and operates at an airflow rate of 5 liters per minute. The concentration of spores per cubic meter was determined by enumerating particles in the *P. chrysogenum* spore size range (1.8–3.5 μm). Background levels of particles

in this size range were obtained prior to release of spores. The airflow through the room was set at approximately 4.2 cubic meters per minute during aerosolization of the spores. Initial contamination of each flooring material was achieved by conveying sufficient spores into the room to obtain an average room airborne concentration of 10^5 spores/m^3 for 15 minutes. After 15 minutes, the Pitt 3 and the room air handling system were turned off. A minimum of 2 hours was allowed for the spores to settle. Surface sampling was then performed to quantify the level of contamination on the flooring material. This protocol resulted in a surface contamination level of approximately 10^6 c.f.u./m^2. After 3 walking activity trials were completed at this level of contamination, additional spore loadings were conducted to raise the level of surface contamination to 10^7 c.f.u./m^2. This was accomplished with a series of 4–6 additional spore aerosolization events as described above, each with an average airborne fungal concentration of 10^6 spores/m^3 for 10 minutes. The higher level of surface contamination was quantified by surface sampling before additional walking trials.

2.6 *Spore viability measurements*

The percent viability of *P. chrysogenum* spores released into the room was estimated for each series of experiments. A sample of spores was removed from the Pitt 3 after aerosol generation and placed in filtered Isoton II solution (Beckman Coulter Inc., Miami, Florida, USA). After sonication of the sample for 10 minutes using a Branson 1200 sonicator (Branson Ultrasonics Corp., Danbury, Connecticut, USA), the total concentration of spores per ml was determined using a Coulter Multisizer II electronic particle counter (Beckman Coulter, Inc.). The number of culturable spores per ml was determined by serial dilution and spread plating of the spore suspension onto triplicate MEA plates. After incubation of plates and enumeration of colonies, the average of the triplicate plates was determined and the percentage of culturable spores released in each trial was estimated.

2.7 *Assessment of flooring contamination*

A quantification method for floor surface contamination was developed for this study. After installation and prior to contamination of each flooring material, pre-cut patches (5.7 × 5.7 cm) of identical flooring material were placed in the room on the flooring surface in 6 groups. Prior to each human activity trial, 1 patch was randomly selected from each group for analysis. These 6 patches were aseptically collected and placed into individual sterile stomacher bags (Tekmar Co., Cincinnati, Ohio, USA). A total of 50 ml of 0.01M potassium phosphate buffer (pH 7.2) with Tween 20 (0.05% final concentration, Sigma Chemical Co., St. Louis, Missouri, USA) was added to each bag and the samples were stomached for 1 minute using a Stomacher 80 mixer (Tekmar Co.). Samples were cultured either by concentration of the buffer onto duplicate sterile mixed cellulose ester membrane filters (Gelman Sciences, Ann Arbor, Michigan, USA) which were placed onto MEA, or serial dilution of the buffer and spread plating in duplicate onto MEA. Samples were incubated at 23 °C for 3 days and the number of c.f.u. were recorded. The mean number of c.f.u. from duplicate plates was used to calculate the number of c.f.u. per patch and c.f.u./m^2 of carpet area for each sample.

2.8 *Air sampling*

The airborne concentrations of culturable and total *P. chrysogenum* spores were measured immediately before and after each walking or cleaning activity. Culturable airborne spore concentrations were measured using Andersen single-stage impactor samplers (Graseby Andersen, Inc., Atlanta, Georgia, USA) supplied with 40 ml MEA plates. Total spores were measured using Burkard personal samplers (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, England, UK) containing a prepared glass slide as the impaction surface. One single-stage Andersen sampler and one Burkard sampler were placed at each of three heights (floor level, 1 meter and 1.5 meters) in the center of the room. All samplers were activated simultaneously from outside the room. The Andersen samplers were operated at an airflow rate of 28.3 liters per minute and the Burkard samplers were operated at 10 liters per minute. Variable sampling times (range: 10 seconds to 5 minutes) were predetermined by the expected airborne concentration to avoid overloading of the sampler surfaces. Background sampling prior to walking was performed at all three heights. Following background sampling, a technician entered the room and replaced the Andersen samplers with unexposed samplers and replaced the exposed Burkard sampler slides with unexposed slides. Walking for 1 minute was then initiated. Immediately after walking, air samples were taken with the Andersen and Burkard samplers as described above. The technician collected

the exposed slides and Andersen samplers and left the room. In the laboratory, the agar plates were aseptically removed from the Andersen samplers and incubated as described above.

Following walking trials with the higher contamination levels, a cleaning activity was performed. Mopping was conducted with the sheet vinyl flooring and vacuuming was conducted with the loop and cut pile carpet flooring. For the mopping, a cotton string mop was wetted with tap water and wrung to release excess water, the wetted mop was then used by the technician to mop the floor of the room for 1 minute. A residential upright vacuum cleaner (Hoover Legacy II, 7.5 amp, The Hoover Company, North Canton, Ohio, USA) was fitted with a new polyethylene vacuum bag (Hysurf^{TM}, E.I DuPont De Nemours & Co., Inc., Richmond, Virginia, USA) and the contaminated carpet was vacuumed for 1 minute.

In addition to background sampling, field audit blank (FAB) samples were collected during each experiment for the Andersen samplers to determine the number of c.f.u. that were deposited by gravitational settling on the uncovered agar sampling surface prior to activation of the samplers. FAB samples consisted of an Andersen sampler placed in the room with the other samplers (1 meter height) but not operated during the pre- and post-activity sampling periods.

2.9 *Data analysis*

Colony counts of *P. chrysogenum* obtained from the Andersen samples were adjusted using the positive hole correction method (Andersen, 1958) and converted to the number of c.f.u./m^3 of air sampled. The Burkard slides were mounted by staining with lacto phenol cotton blue (Medical Chemical Corp., Santa Monica, California, USA) and adding a glass cover slip. Total spores with the morphological characteristics of *Penicillium* spores were enumerated under 400$\times$ total magnification. The number of spores per sample was converted to spores/m^3 of air sampled. Background levels of c.f.u./m^3 and spores/m^3 measured prior to human activity were subtracted from post-activity measurements. For surface samples, *P. chrysogenum* colony counts obtained from duplicate plates were averaged and converted to c.f.u. per patch and c.f.u./m^2. All air and surface sample data were $\log_{10}$ transformed and multifactor analysis of variance procedures were performed. A general linear model was performed to analyze airborne *P. chrysogenum* concentration data. Four factors were examined: surface contamination level (10^6 c.f.u./m^2 and 10^7 c.f.u./m^2), type of flooring material (vinyl tile, loop pile carpet, and cut pile carpet), sampler type (Andersen and Burkard samplers) and sampler location (floor, 1 meter and breathing zone). In addition, the interaction between all possible pairs of factors was examined.

3. Results

3.1 *Flooring material contamination*

Precision of the patch sample method was assessed by analyzing in triplicate a single patch taken for each experiment conducted during the sheet vinyl activity trials. The mean coefficient of variation was 1.09% $\pm$ 0.29% (1 standard error) for replicate analyses of the same sample (data not shown).

Surface fungal spore measurements for the 3 flooring materials contaminated at the 10^6 c.f.u./m^2 and 10^7 c.f.u./m^2 contamination levels are shown in Table 2. Uniform distribution of fungal spores on the flooring materials was achieved and there were no significant differences in c.f.u./m^2 between the 6 sample locations spaced throughout the room (α = 0.05). Although it was desired that the contamination levels of viable fungi for all the flooring materials be equivalent, statistically significant differences were observed. At the 10^6 c.f.u./m^2 contamination level, the initial fungal contamination level (trial 1) on the sheet vinyl patches was significantly lower than on the loop pile and cut pile carpet patches (P = 0.009). For the 10^7 c.f.u./m^2 contamination level trials, the initial fungal concentration on residential carpet patches was significantly lower than the other 2 flooring materials (P = 0.001).

3.2 *Airborne spore concentrations after walking*

The percentage of potentially available settled surface spores actually re-suspended by walking or cleaning from any of the flooring materials was very low (Table 3). It was also noted that re-suspended spore levels consistently fell after each subsequent walking disturbance on the various flooring materials (Figures 2 and 3).

Data collected with the sheet vinyl and loop pile carpet contaminated at the 10^6 c.f.u./m^2 level during walking showed low culturable airborne fungal spore concentrations (Figure 2A). Some measurements were at or below the lower detection limit (7 c.f.u./m^3 for

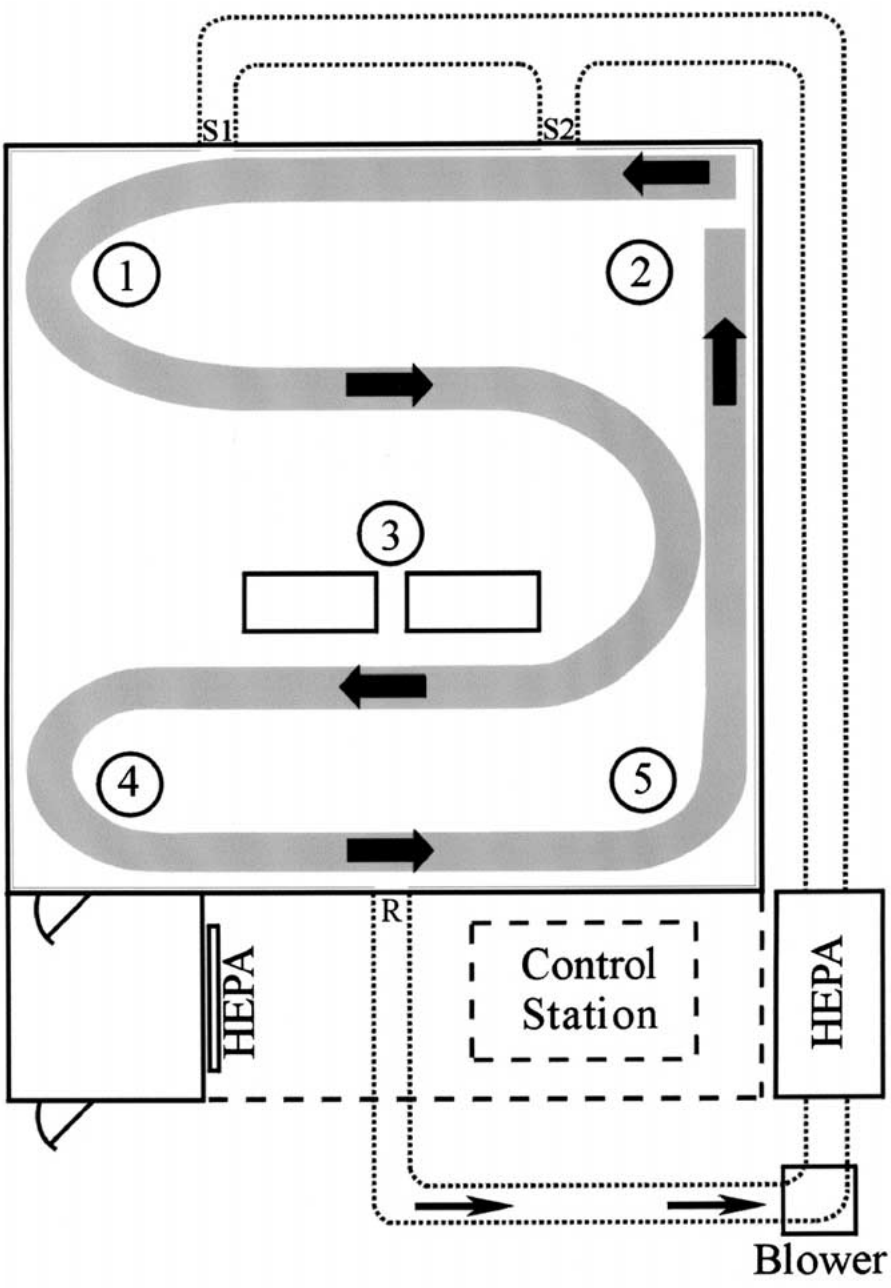

Figure 1. Diagram of the experimental room. The shaded area represents the walking path for trials on contaminated flooring materials. The room air handling system delivers HEPA-filtered air into the room through 2 supply registers (S1 and S2) and exits through one return register (R). Sampling stands (locations 1–5) support temperature and relative humidity probes. Air samplers were located in the center of the room (rectangular boxes).

Table 2. Measurements of surface contamination levels on flooring materials prior to each of 3 walking trials at 2 contamination levels. Data are the mean of 6 floor patches (± 1 standard error, in parentheses)

Flooring material	Contamination level (c.f.u./m^2)	Culturable *P. chrysogenum* on Floor Patches (c.f.u./m^2)		
		Trial 1	Trial 2	Trial 3
Vinyl tile	10^6	$\mathbf{1.36 \times 10^6}$	$\mathbf{1.57 \times 10^6}$	$\mathbf{1.98 \times 10^6}$
		(2.29×10^5)	(1.20×10^5)	(1.15×10^5)
Commercial carpet		$\mathbf{2.04 \times 10^6}$	$\mathbf{1.97 \times 10^6}$	$\mathbf{1.96 \times 10^6}$
		(7.46×10^4)	(7.87×10^4)	(1.13×10^5)
Residential carpet		$\mathbf{2.15 \times 10^6}$	$\mathbf{2.29 \times 10^6}$	$\mathbf{2.20 \times 10^6}$
		(9.24×10^4)	(1.33×10^5)	(1.19×10^5)
Vinyl tile	10^7	$\mathbf{8.22 \times 10^7}$	$\mathbf{5.64 \times 10^7}$	$\mathbf{8.95 \times 10^7}$
		(5.09×10^6)	(1.08×10^7)	(5.46×10^6)
Commercial carpet		$\mathbf{8.04 \times 10^7}$	$\mathbf{7.20 \times 10^7}$	$\mathbf{8.80 \times 10^7}$
		(2.87×10^6)	(4.18×10^6)	(4.25×10^6)
Residential carpet		$\mathbf{5.89 \times 10^7}$	$\mathbf{7.53 \times 10^7}$	$\mathbf{7.88 \times 10^7}$
		(3.08×10^6)	(4.15×10^6)	(6.93×10^6)

Table 3. Estimates of the percent of *P. chrysogenum* spores aerosolized in the experimental room as a result of walking on fungal-contaminated flooring materials

Flooring material	Contamination level (c.f.u./m^2)	*P. chrysogenum* spores aerosolized (%)		
		Trial 1	Trial 2	Trial 3
Vinyl tile	10^6	0.0040	0.0026	$<$0.0008
Commercial carpet		0.00015	0.00008	0.00009
Residential carpet		0.39	0.23	0.076
Vinyl tile	10^7	0.0043	0.0037	0.0007
Commercial carpet		0.0043	0.0033	0.0011
Residential carpet		$\geq$0.37	0.18	0.22

a 5 minute sample). Measurements with the cut pile carpet showed higher airborne culturable concentrations, ranging from 10^2 to 10^3 c.f.u./m^3. Total spore measurements obtained with the Burkard personal slide impactor (Figure 2B) were higher than culturable measurements (Figure 2A) for all flooring materials. Data from walking trials conducted at the 10^7 c.f.u./m^2 contamination level showed a greater release of spores (Figure 3). A similar trend to that observed at the 10^6 c.f.u./m^2 contamination level was demonstrated for both culturable (Figure 3A) and total (Figure 3B) airborne spore measurements, with the highest number of spores aerosolized by walking on the cut pile carpet. Airborne concentrations of spores dispersed from sheet vinyl and loop pile carpet were comparable. Culturable fungal concentrations after the first walking trial with cut pile carpet exceeded the upper detection limit of the Andersen sampler (9.39×10^4 c.f.u./m^3 for a 1 minute sample).

A general linear model was performed to analyze airborne *P. chrysogenum* concentration data. A statistically significant difference was noted with respect to flooring material ($P = 0.000$). The cut pile carpet data were significantly higher than the other flooring materials for both the 10^6 c.f.u./m^2 (Figures 2A and 3A) and the 10^7 c.f.u./m^2 (Figures 2B and 3B) contamination levels. Further analysis using pooled data with the Andersen sampler showed no difference between the concentrations of airborne culturable *P. chrysogenum* with sheet vinyl and loop pile carpet at the 10^6 c.f.u./m^2 ($P = 0.17$) or 10^7 c.f.u./m^2 ($P = 0.51$) contamination levels. Burkard sampler data also indicated no significant difference in the numbers of airborne spores/m^3 at the 10^6 c.f.u./m^2 contamination

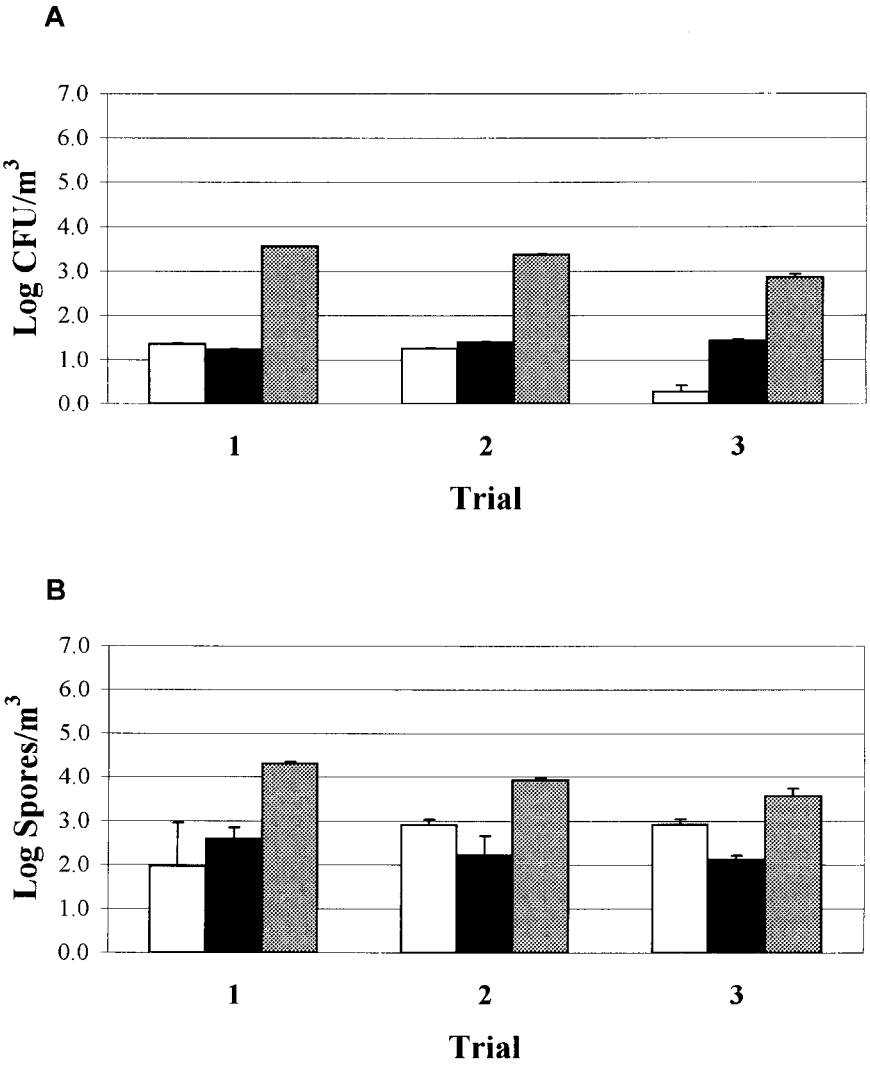

Figure 2. Concentrations of airborne *P. chrysogenum* measured with Andersen impactor samplers (A) and Burkard personal impactor samplers (B) after 3 walking trials on flooring materials contaminated with *P. chrysogenum* spores at a level of 10^6 c.f.u./m^2. Flooring materials tested were vinyl tile (white bars), commercial carpet (black bars) and residential carpet (gray bars). Bar heights represent the mean of 3 samples $\pm$ 1 standard error.

level ($P = 0.45$). However, total airborne spore concentrations were significantly higher for the loop pile than for the sheet vinyl at the 10^7 c.f.u./m^2 contamination level ($P = 0.0037$; Figure 3B).

A significant difference was observed in airborne *P. chrysogenum* concentrations with respect to contamination level ($P = 0.000$). The airborne counts were greater with higher surface loading. There was a significant difference in airborne concentrations between sampler types ($P = 0.000$), with the Burkard data higher than the Andersen data for both loading concentrations. There was no significant difference between sampler locations ($P = 0.076$) although the 1-meter location data were the highest (mean $\log_{10}$ 3.253), followed by the floor level (mean $\log_{10}$ 3.168) and the breathing zone (mean $\log_{10}$ 3.014) locations.

The study design permitted the cleaning activity (mopping or vacuuming) to be conducted once per flooring material. Therefore, the observations were estimates based on a single trial. Vacuuming loop pile carpet resulted in a 17-fold increase in culturable *P. chrysogenum* over measurements obtained after the 3rd walking trial, while the data for cut pile carpet

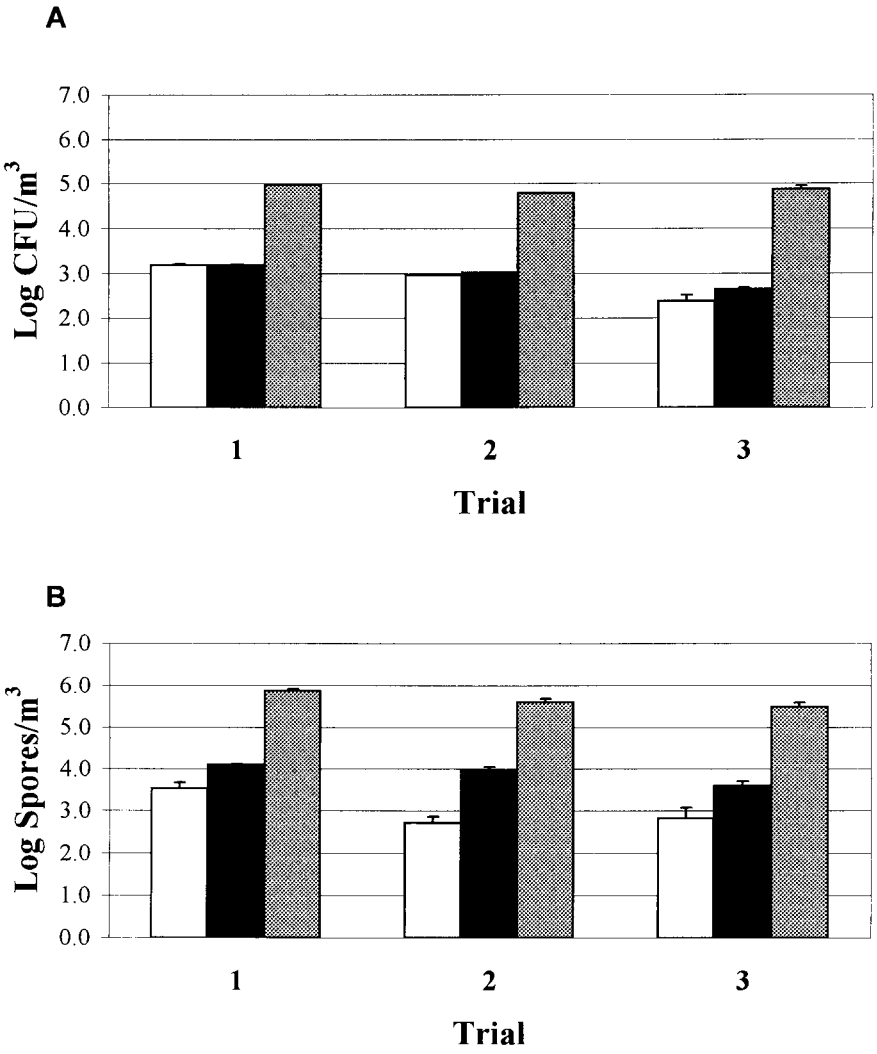

Figure 3. Concentrations of airborne *P. chrysogenum* measured with Andersen impactor samplers (A) and Burkard personal impactor samplers (B) after 3 walking trials on flooring materials contaminated with *P. chrysogenum* spores at a level of 10^7 c.f.u./m^2. Flooring materials tested were vinyl tile (white bars), commercial carpet (black bars) and residential carpet (gray bars). Bar heights represent the mean of 3 samples $\pm$ 1 standard error.

showed a 2-fold increase. The impact of wet mopping was approximately a $\geq$179-fold increase over that observed with the 3rd walking trial, however a precise measurement could not be obtained due to overloading of the Andersen sampler. In contrast to culture data, total spore measurements showed that mopping of the vinyl tile produced a 40-fold increase in the concentration of airborne spores compared to the effect of walking. For cut pile and loop pile carpets, vacuuming did not increase the airborne concentrations over that observed with walking.

3.3 *Physical measurements and spore viability data*

Mean temperature and relative humidity measurements in the room ranged from 73.4°F to 77.2°F and 22.3% to 33.4%, respectively, during the 18 walking trials. All FAB samples taken to assess if *P. chrysogenum* was deposited by gravitational settling onto the uncovered agar sampling surface prior to activation of the Andersen samplers were negative. Estimates of *P. chrysogenum* spore viability averaged 81% $\pm$ 2% (1 standard error; n = 17), with a range of 64–97%.

4. Discussion

A reliable, repeatable, quantitative assessment of surface contamination was needed to evaluate the dispersal of fungal spores from floor materials. The patch sampling method developed in this project provided precise measurements for all three materials tested. The use of the Pitt 3 and acoustic vibration provided a uniform contamination of the materials through the HVAC system of the experimental room.

The higher airborne *P. chrysogenum* data obtained in the cut pile carpet experiments compared to the other two flooring materials supports data obtained in recently published small scale laboratory work (Kildeso, 1999) using a falling weight onto artificially soiled carpets to re-suspend dusts; however, the same effect was not observed in field studies (Kildeso, 1999).

The reasons for the differences in data collected between the cut pile carpet and the other two flooring materials were not determined in this study but physical attributes associated with cut pile carpets may have contributed. For example, the majority of the fibers in cut pile carpet are oriented vertically, allowing for the settling of spores onto fiber tips and in the interstitial spaces at the top of the carpet tuft, which could then be readily distributed by walking. In addition, there is substantially more vertical compression of cut pile fibers associated with a footfall that could create a greater physical disturbance of settled spores.

For all flooring materials there was no difference between sampler heights. The absence of a significant vertical concentration gradient indicates that the *P. chrysogenum* spores were dispersed quickly and uniformly throughout the test room.

The difference between the data collected with the two types of aerobiological samplers may be explained by the fact that the Andersen sampler measures culturable airborne concentrations and the Burkard sampler measures total spores (culturable and non-culturable spores).

For all flooring materials, the numbers of airborne spores decreased over the 3 walking trials at each contamination level. It was possible that fewer spores were available due to redistribution associated with repeated passes over the walking path. However, because a small percentage of spores became airborne and most of the settled spores remained in place, another mechanism may have been involved. *P. chrysogenum* spores are 2–3 μm in diameter and Thatcher and Layton (1995) stated that particles $\leq$5 μm diameter are not easily re-suspended from a surface. Electrostatic attraction may contribute to this effect (Morton and Hearle, 1962; Price et al., 1990; Dart and Obendorf, 2000), but the induced charge properties of *P. chrysogenum* spores are unknown.

In conclusion, results from this research project demonstrate that: (i) the type of flooring material and the settled biocontamination level affect the magnitude of airborne dispersal of fungal spores after walking activity, (ii) fungal spores dispersed from flooring materials were entrained rapidly and uniformly throughout the room, (iii) a low percentage of fungal spores present on flooring materials becomes aerosolized by walking activity despite very high surface contamination, and (iv) repeated disturbances have an impact in reducing the level of particles aerosolized by subsequent activity.

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